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Results: Exposure of cartilage to blood at all sides, both articular surface and cutting edges, led to a decrease in proteoglycan synthesis rate of −95% and an increase in proteoglycan release of +67%. These effects were less outspoken when the cartilage was exposed to only the articular surface: 56% and +13% for proteoglycan synthesis rate and -release respectively.
Conclusions: *In vitro* exposure of cartilage to blood at the articular surface alone leads to less severe effects on the proteoglycan synthesis rate and - release than when cartilage explants are exposed at all sides. This is probably part of the explanation why blood-induced cartilage damage after an experimentally induced haemarthros *in vivo* is less severe compared to the *in vitro* effects of blood on cartilage. Irrespectively, blood has devastating effects on articular cartilage, and in this respect it is important to prevent (traumatic) joint haemorrhages and if they occur, to treat them properly. Additionally this study demonstrates that results of cartilage tissue explant cultures, exposed at all sides to culture medium and additions should be interpreted with caution.

153 ANTI-INFLAMMATORY PROPERTIES OF STIGMASTEROL IN CARTILAGE: NEW INSIGHTS

O.H. Gabay Jr[1], C. Sanchez Jr.[2], F. Chevy Sr.[3], M. Breton Sr.[3], G. Nourissat Jr.[1], C. Wolf Sr.[3], F. Berenbaum Sr.[1]. [1]*Paris Universitas – UPMC, UMR CNRS 7079, APHP St Antoine hospital, Paris, FRANCE,* [2]*Cartilage and Bone Metabolism Research Unit, CHU Sart Tilman, University of Liege, BELGIUM,* [3]*Paris Universitas – Faculty of Medicine Paris VI St Antoine inserm U538, Paris, FRANCE*

Purpose: Although most studies have focused on the cholesterol-lowering activity of stigmasterol, other biological actions have been ascribed to this plant sterol compound, one of which is a potential anti-inflammatory effect.
The objective of this study is to investigate the effects of stigmasterol, a plant sterol, on the inflammatory mediators and metalloproteinases production by chondrocytes.
Methods: We used a model of newborn mouse costal chondrocytes and human OA chondrocytes in primary culture stimulated or not with IL1beta (10 ng/ml), 6 or 18 h. Cells were pre-incubated for 48 h with stigmasterol (20 μg/ml) dissolved in ethanol 0.1%. Cells pre-incubated with ethanol alone served as controls. We investigate first the presence of stigmasterol in chondrocyte, compared to other phytosterols. We investigated then the role of stigmasterol on different genes expression involved in inflammation (Il-6) and cartilage degradation (MMP-3,-13, ADAMTS -4, -5, type II collagen, aggrecan) by quantitative RT-PCR (Light Cycler®, Roche) and on the production of MMP-3 and PGE_2 by specific immuno-enzymatic assays. We then looked at the role of stigmasterol on NF-kappaB activation by western blot, using an anti-IkappaBalpha antibody.
Results: At 18 h, Il-1beta treatment induced MMP-3 (×25), MMP-13 (×6), ADAMTS-4 (×2.5), ADAMTS-5 (×2) mRNA expression as well as MMP-3 (×14) and PGE_2 (151%) synthesis and reduced type II collagen (86%) and aggrecan (73%) mRNA expression by chondrocytes. Stigmasterol pre-incubation significantly decrease this 18 h Il-1beta-stimulated MMP-3 (by 82%), MMP-13 (by 85%) and ADAMTS-4 (by 41%) gene expression and MMP-3 production (by 33%) in chondrocytes, but no effect was observed on Il-6, ADAMTS-5, type II collagen and aggrecan gene expression Il-1beta-stimulated. Moreover, stigmasterol pre-incubation decreased by 50% the Il-1beta-stimulated PGE_2 production by OA chondrocytes. Finally, stigmasterol was capable to counteract the Il-1beta-induced NF-kappa B pathway.
Conclusions: This in-vitro study shows that stigmasterol inhibits some pro-inflammatory and pro-degradative mediators involved in the OA-induced cartilage degradation process, in part through the inhibition of the NF-kappa B pathway. These promising results should lead now to ex-vivo and in vivo investigations.

154 MECHANICAL STRESS ENHANCES THE CARTILAGE-SPECIFIC GENE EXPRESSION OF RAT CHONDROCYTES *VIA* AUTOCRINE LOOP OF INTERLEUKIN-4

S. Shioji, S. Imai, K. Ando, N. Okumura, T. Mimura, K. Uenaka, K. Nishizawa. *Shiga University of Medical Science, Otsu, JAPAN*

Purpose: Interleukin-4 (Il-4) has been long suggested to protect the articular cartilage. For instance, Il-4 inhibits catabolism of cartilage matrix *via* regulating activities of matrix metalloproteinases. Yet, it remained long unclear whether Il-4 directly acts on the chondrocytes. However, accumulating molecular biological studies suggest that Il-4 is intimately involved in the molecular events that regulate the function of chondrocyets. In turn, mechanical stress has long been known to influence the chondrocyte function. The aim of this study was to investigate whether Il-4 plays any role in regulating the molecular function of chondrocyte in response to the mechanical stress. The present study used the 3-dimentionally (3-D) embedded chondrocytes to examine the effect of mechanical stress or Il-4 on the expression of type II collagen (Col.2) and aggrecan (AGC) mRNA.
Methods: Chondrocytes were freshly isolated from rat articular cartilage. On reaching confluence, the cells were 3-D embedded in type I collagen scaffold. The cell-seeded scaffold was cultured either under mechanical stress (MS group) or with Il-4 (Il-4 group). The mechanical stress was a cyclic compression at 5% compression, 0.33 Hz for 1hours, and the Il-4 concentrations were 10 ng/ml. The 3-D embedded chondrocytes without mechanical stress nor Il-4 served as negative control (NS group). Real-time PCR was performed for Col.2, AGC, and GAPDH, as an internal control at 1, 6, 12, and 24 hours after the application of mechanical stress or Il-4. Statistical significance was evaluated by modified one-factor analysis of variance (ANOVA). P values <0.05 were considered significant.
Results: Expression of AGC and Col.2 was significantly upregulated in the MS group as well as in the Il-4 group when compared with that of the NS group. Intriguingly, expression of Il-4 gene was also upregulated when chondrocytes were mechanically stressed. The mechanical stress-induced upregulation of the matrix synthesis was attenuated when Il-4 inhibitor was applied.
Conclusions: The present results show that Il-4 and MS influence the matrix synthesis of the 3-D embedded chondrocytes. Since Il-4 expression is also up-regulated by mechanical stress, Il-4 is likely to be involved in the molecular events. In turn, the inhibition of the mechanical stress-related enhancement of matrix synthesis by the Il-4 inhibitor strongly supports our hypothesis that the mechanical stress regulates the matrix synthesis *via* autocrine loop of Il-4.

155 DIFFERENTIAL GENE-EXPRESSION PROFILES OF ARTICULAR CARTILAGE TISSUE DERIVED FROM THE METACARPAL, SHOULDER AND KNEE JOINTS OF SKELETALLY IMMATURE AND MATURE COWS

N. Shintani, S. Nüssli, E.B. Hunziker. *DST Research Center, Department of Clinical Research, University of Bern, Bern, SWITZERLAND*

Purpose: Articular cartilage is characterized by a highly specialized structure and composition, which reflect its unique functions within the synovial joint. But these morphological and biochemical peculiarities are achieved at a high cost, in that traumatized or diseased tissue possesses a very limited capacity to repair spontaneously. To overcome this intrinsic biological problem, many investigators are now attempting to engineer articular cartilage using cell populations of diverse origin and sundry growth factors. But ultimate success in these endeavours will depend upon a thorough knowledge of the biology of articular cartilage tissue. We postulate that the biochemical and biomechanical characteristics of articular cartilage are joint specific. If this tenet is borne out, then the finding could have an important impact on the approach to articular cartilage repair. To verify our hypothesis, we investigated the gene-expression profiles of articular cartilage tissue that was derived from three different joints of skeletally immature and mature bovine cows.
Methods: Articular cartilage was harvested from the metacarpal (basal region), shoulder (humeral head) and knee (tibial plateau) joints of 3- to 4-month-old bovine calves (n = 5) and 15- to 20-month-old bovine cows (n = 5) within 24 hours of slaughtering. The tissue was first pulverized in a freezer-mill and the RNA was then isolated. The RNA derived from each sample was reversed transcribed and then subjected to a real-time PCR analysis. The primers and the probes for collagen types I, II, IX, X and XI, aggrecan, COMP, Sox9 and 18S rRNA were generated as previously described (Shintani et al., Arthritis Rheum 56:1869, 2007). The gene-expression levels were calculated relative to those in an arbitrary calibrator.
Results: In immature articular cartilage, the patterns of expression of the marker genes were comparable in each of the three joints. The sole exception was that for type I collagen, which was expressed at a higher level in the metacarpal than in the shoulder joint; there was no significant difference between the levels in the metacarpal and knee joints. During the process of maturation, the gene-activity profiles for articular cartilage changed dramatically in each of the three joint types. Generally, those for collagen types IX and XI decreased with age. However, joint-specific